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L1: Entry 1 of 1 File: USPT Sep 4, 2001

US-PAT-NO: 6284494
DOCUMENT-IDENTIFIER: US 6284494 B1

TITLE: Methods and compositions for synthesis of oligosaccharides using mutant glycosidase enzymes

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
MacKenzie; Lloyd	Vancouver			CA
Wang; Qingping	Kirkland			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The University of British Columbia	Vancouver			CA	03

APPL-NO: 09/ 091272 [PALM]
DATE FILED: September 29, 1998

PARENT-CASE:
This application is a U.S. National Phase, filed under 35 USC .sctn. 371, of PCT/CA96/00841, which is a continuation-in-part of U.S. patent application Ser. No. 08/571,175 filed Dec. 12, 1995, now U.S. Pat. No. 5,716,812.

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/CA96/00841	December 12, 1996	WO97/21822	Jun 19, 1997	Sep 29, 1998	Sep 29, 1998

INT-CL: [07] C12 P 19/44, C12 P 19/12, C12 N 9/24, C12 N 9/26, C12 N 9/42

US-CL-ISSUED: 435/74; 435/100, 435/200, 435/201, 435/209
US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209

FIELD-OF-SEARCH: 435/74, 435/100, 435/200, 435/201, 435/209, 435/440

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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☐	4918009	April 1990	Nilsson	435/73
☐	5246840	September 1993	Nilsson	435/101
☐	5372937	December 1994	Nilsson	435/74

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ART-UNIT: 162

PRIMARY-EXAMINER: Slobodyansky; Elizabeth

ABSTRACT:

Mutant glycosidase enzymes are formed in which the normal nucleophilic amino acid within the active site has been changed to a non-nucleophilic amino acid. These enzymes cannot hydrolyze disaccharide products, but which can still form them. Using this enzyme, oligosaccharides are synthesized by preparing a mixture of an .alpha.-glycosyl fluoride and a glycoside acceptor molecule; enzymatically coupling the .alpha.-glycosyl fluoride to the glycoside acceptor molecule to form a glycosyl glycoside product using the mutant glycosidase enzyme; and recovering the glycosyl glycoside product. Particular enzymes include a mutant form of Agrobacterium .beta.-Glucosidase in which the normal glutamic acid residue at position 358 is replaced with an alanine residue.

2 Claims, 3 Drawing figures

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L1: Entry 1 of 1 File: USPT

US-PAT-NO: 6284494
DOCUMENT-IDENTIFIER: US 6284494 B1

TITLE: Methods and compositions for synthesis of oligosaccharides using mutant glycosidase enzymes

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
MacKenzie; Lloyd	Vancouver			CA
Wang; Qingping	Kirkland			CA

US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209

CLAIMS:

What is claimed is:

1. A method for synthesizing an oligosaccharide comprising the steps of:

(a) combining a glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture, said glycosyl donor molecule having a .beta. configuration and said glycoside acceptor molecule having an .alpha. configuration, or vice versa; and

(b) enzymatically coupling the donor molecule to the acceptor molecule using Agrobacterium .beta.-glucosidase in which amino acid 358 has been changed from glutamic acid to an amino acid with a non-carboxylic acid side chain.

2. The method of claim 1, wherein the, amino acid 358 has been changed from glutamic acid to alanine.

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L2: Entry 5 of 6 File: USPT Sep 14, 1999

US-PAT-NO: 5952203
DOCUMENT-IDENTIFIER: US 5952203 A

TITLE: Oligosaccharide synthesis using activated glycoside derivative, glycosyl transferase and catalytic amount of nucleotide phosphate

DATE-ISSUED: September 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
Lougheed; Brenda	Vancouver			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The University of British Columbia	Vancouver			CA	03

APPL-NO: 08/ 835941 [PALM]
DATE FILED: April 11, 1997

INT-CL: [06] C12 P 19/18, C12 P 19/04, C12 N 11/12, C12 N 9/10

US-CL-ISSUED: 435/97; 435/100, 435/101, 435/174, 435/179, 435/193
US-CL-CURRENT: 435/97; 435/100, 435/101, 435/174, 435/179, 435/193

FIELD-OF-SEARCH: 435/72, 435/74, 435/97, 435/100, 435/101, 435/174, 435/179, 435/193

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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5374655	December 1994	Kashem et al.	514/540
5716812	February 1998	Withers et al.	435/74
5750389	May 1998	Elling et al.	435/193

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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WO 94/01540	January 1994	WO	
WO 96/32491	October 1996	WO	
WO 97/21822	June 1997	WO	

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ART-UNIT: 161

PRIMARY-EXAMINER: Naff; David M.

ABSTRACT:

Oligosaccharides are prepared using glycosyl transferase and activated glycosyl derivatives as donor sugars without the use of sugar nucleotides as donor sugars. A reaction mixture composition containing an activated glycoside derivative such as glycosyl fluoride or glycosyl mesylate, an acceptor substrate such as lactose or other oligosaccharide, a glycosyl transferase and a catalytic amount of a nucleotide phosphate or nucleotide phosphate analog is reacted to produce a glycosylated acceptor. In addition to an oligosaccharide, the acceptor substrate may be a monosaccharide, a fluorescent-labeled saccharide or a saccharide derivative such as an aminoglycoside antibiotic. The glycosyl transferase may be immobilized by removing its membrane-binding domain and attaching in its place a cellulose-binding domain. In a preferred embodiment, galactosyl transferase is the glycosyl transferase, .alpha.-D-galactosyl fluoride is the activated glycoside derivative and lactose is the acceptor substrate.

19 Claims, 4 Drawing figures

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L2: Entry 5 of 6 File: USPT

US-PAT-NO: 5952203
DOCUMENT-IDENTIFIER: US 5952203 A

TITLE: Oligosaccharide synthesis using activated glycoside derivative, glycosyl transferase and catalytic amount of nucleotide phosphate

DATE-ISSUED: September 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
Lougheed; Brenda	Vancouver			CA

US-CL-CURRENT: 435/97; 435/100, 435/101, 435/174, 435/179, 435/193

CLAIMS:

What is claimed is:

1. A composition useful for the formation of glycosidic linkages comprising an admixture of an activated glycoside derivative, a glycosyl transferase, an acceptor substrate, and a catalytic amount of a nucleotide phosphate or a nucleotide phosphate analog.

2. A composition in accordance with claim 1, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is a disaccharide.

3. A composition in accordance with claim 1, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is lactose.

4. A process for using an activated glycoside derivative to glycosylate an acceptor substrate, comprising:

(a) admixing in an aqueous medium said activated glycoside derivative, said acceptor substrate, a glycosyl transferase, and a catalytic amount of a member selected from the group consisting of a nucleotide phosphate and a nucleotide phosphate analog, to form an aqueous reaction mixture; and

(b) maintaining said aqueous reaction mixture at a pH value of about 5 to about 10, and at a temperature ranging from between about freezing to about a temperature at which said glycosyl transferase denatures for a period of time sufficient for glycosylation of said acceptor to occur, thereby forming a glycosylated acceptor.

5. A process in accordance with claim 4, wherein said activated glycoside derivative is a glycosyl fluoride.

6. A process in accordance with claim 4, wherein said activated glycoside derivative is a glycosyl mesylate.

7. A process in accordance with claim 4, further comprising the step of

(c) recovering said glycosylated acceptor.

8. A process in accordance with claim 4, wherein said glycosyl transferase is a member selected from the group consisting of .alpha.-sialyl transferases, .alpha.-glucosyl transferases, .alpha.-galactosyl transferases, .alpha.-fucosyl transferases, .alpha.-mannosyl transferases, .alpha.-xylosyl transferases, .alpha.-N-acetyl hexosaminyl transferases, .beta.-sialyl transferases, .beta.-glucosyl transferases, .beta.-galactosyl transferases, .beta.-fucosyl transferases, .beta.-mannosyl transferases, .beta.-xylosyl transferases, and .beta.-N-acetyl hexosaminyl transferases.

9. A process in accordance with claim 4, wherein said aqueous medium is a buffered aqueous medium.

10. A process in accordance with claim 4, wherein said acceptor substrate is selected from the group consisting of an oligosaccharide, a monosaccharide, a fluorescent-labeled saccharide and a saccharide derivative.

11. A process in accordance with claim 10, wherein said saccharide derivative is an aminoglycoside antibiotic.

12. A process in accordance with claim 10, wherein said oligosaccharide is lactose.

13. A process in accordance with claim 10, wherein said fluorescent-labeled saccharide is selected from the group consisting of an FITC-lactose, FCHASE-lactose, FITC-galactose and FCHASE-galactose.

14. A process in accordance with claim 5, wherein said glycosyl fluoride is a member selected from the group consisting of .alpha.-galactosyl fluoride, .alpha.-mannosyl fluoride, .alpha.-glucosyl fluoride, .alpha.-fucosyl fluoride, .alpha.-xylosyl fluoride, .alpha.-sialyl fluoride, .alpha.-N-acetylglucosaminyl fluoride, .alpha.-N-acetylgalactosyl fluoride, .beta.-galactosyl fluoride, .beta.-mannosyl fluoride, .beta.-glucosyl fluoride, .beta.-fucosyl fluoride, .beta.-xylosyl fluoride, .beta.-sialyl fluoride, .beta.-N-acetylglucosaminyl fluoride and .beta.-N-acetylgalactosyl fluoride.

15. A process in accordance with claim 4, wherein said glycosyl transferase is a member selected from the group consisting of .alpha.-sialyl transferases, .alpha.-glucosyl transferases, .alpha.-galactosyl transferases, .alpha.-mannosyl transferases, .alpha.-fucosyl transferases, .alpha.-xylosyl transferases, .alpha.-N-acetyl hexosaminyl transferases, .beta.-sialyl transferases, .beta.-glucosyl transferases, .beta.-galactosyl transferases, and .beta.-N-acetyl hexosaminyl transferases.

16. A process in accordance with claim 4, wherein said glycosyl transferase is immobilized on a solid support.

17. A process in accordance with claim 4, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is a disaccharide.

18. A process in accordance with claim 4, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .beta.-D-galactosyl fluoride and said acceptor substrate is lactose.

19. A process in accordance with claim 4, wherein said temperature range is between about 0.degree. C. to about 40.degree. C.

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L2: Entry 4 of 6 File: USPT Mar 20, 2001

US-PAT-NO: 6204029
DOCUMENT-IDENTIFIER: US 6204029 B1

TITLE: Glycosylated acceptor synthesis catalyzed by glycosyl transferase and nucleotide phosphate sugar-dependent enzyme

DATE-ISSUED: March 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
Lougheed; Brenda	Vancouver			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The University of British Columbia	Vancouver			CA	03

APPL-NO: 09/ 057863 [PALM]
DATE FILED: April 9, 1998

PARENT-CASE:
This application is a Continuation-in-Part of U.S. patent application Ser. No. 08/835,941 filed Apr. 11, 1997, now U.S. Pat. No. 5,952,203.

INT-CL: [07] C12 P 19/18, C12 P 19/04, C12 N 11/12, C12 N 9/10

US-CL-ISSUED: 435/97; 435/100, 435/101, 435/174, 435/179, 435/193
US-CL-CURRENT: 435/97; 435/100, 435/101, 435/174, 435/179, 435/193

FIELD-OF-SEARCH: 435/89, 435/91.1, 435/97, 435/174, 435/177, 435/180, 435/100, 435/101, 435/179, 435/193

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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5374655	December 1994	Kashem et al.	514/540
5716812	February 1998	Withers et al.	435/74
5750389	May 1998	Elling et al.	435/193

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 92/16640	October 1992	WO	
WO 94/01540	January 1994	WO	
WO 96/32491	October 1996	WO	
WO 97/21822	June 1997	WO	

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Paulson et al., J. Biol. Chem. 264:17615-17618 (1989).
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Dabkowski et al., Transplant Proc. 25:2921 (1993).
Yamamoto et al., Nature 345:229-233 (1990).
Palcic et al., Carbohydrate Res. 190:1-11 (1989).
Prieels et al., J. Biol. Chem. 256:10456-10463 (1981).
Nunez et al., Can. J. Chem. 59:2086-2095 (1981).
Dumas et al., Bioorg. Med. Letters 1:425-428 (1991).
Kukowska-Latallo et al., Genes and Development 4:1288-1303 (1990).
Mollicone et al., Eur. J. Biochem. 191:169-176 (1990).
Stagljar et al., Proc. Natl. Acad. Sci. USA 91:5977-5981 (1994).
Heesen et al., Eur. J. Biochem. 224:71-79 (1994).
Nagata et al., J. Biol. Chem. 267:12082-12089 (1992).
Smith et al., J. Biol Chem. 269:15162-15171 (1994).
Homa et al., J. Biol Chem. 268:12609-12616 (1993).
Hull et al., BBRC 176:608-615 (1991).
Ihara et al., J. Biolchem. 113:692-698 (1993).
Shoreiban et al., J. Biol. Chem. 268:15381-15385 (1993).
Bierhuizen et al., Proc. Natl. Acad. Sci. USA 89:9326-9330(1992).
Rajput et al., Biochem J. 285:985-992 (1992).
Hayashi et al., Chem. Lett. 1747-1750 (1984).

ART-UNIT: 161

PRIMARY-EXAMINER: Naff; David M.

ABSTRACT:

Glycosylated acceptors are prepared using glycosyl transferase and activated glycosyl derivatives as donor sugars without the use of sugar nucleotides as donor sugars. A reaction mixture composition containing an activated glycoside derivative such as glycosyl fluoride or glycosyl mesylate, an acceptor substrate such as lactose or other oligosaccharide, a glycosyl transferase and a catalytic amount of a nucleotide phosphate or nucleotide phosphate analog is reacted to produce the glycosylated acceptor. The acceptor substrate may also be a monosaccharide, a fluorescent-labeled saccharide or a saccharide derivative such as an aminoglycoside antibiotic. The glycosyl transferase may be immobilized by removing its membrane-binding domain and attaching in its place a cellulose-binding domain. In another embodiment, a glycosylated acceptor is formed by making a nucleotide phosphate glycoside in situ in a steady state concentration. This process is carried out by admixing in an aqueous medium an activated glycoside derivative, a glycosyl transferase, a member selected from the group consisting of a nucleotide phosphate and a nucleotide phosphate analog, a nucleotide phosphate sugar-dependent enzyme and an acceptor substrate. The glycosyl transferase catalyzes the reaction of the activated glycoside derivative with the nucleotide phosphate or analog to form the nucleotide phosphate glycoside in situ, and the nucleotide phosphate sugar-dependent enzyme catalyzes the reaction of the nucleotide phosphate glycoside with the acceptor substrate to form the glycosylated acceptor.

39 Claims, 16 Drawing figures

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L2: Entry 4 of 6 File: USPT

US-PAT-NO: 6204029
DOCUMENT-IDENTIFIER: US 6204029 B1

TITLE: Glycosylated acceptor synthesis catalyzed by glycosyl transferase and nucleotide phosphate sugar-dependent enzyme

DATE-ISSUED: March 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
Lougheed; Brenda	Vancouver			CA

US-CL-CURRENT: 435/97; 435/100, 435/101, 435/174, 435/179, 435/193

CLAIMS:

What is claimed is:

1. A composition for forming a glycosylated acceptor comprising an admixture of an activated glycoside derivative, a glycosyl transferase altered by mutation, an acceptor substrate, and a catalytic amount of a nucleotide phosphate or a nucleotide phosphate analog.

2. A composition in accordance with claim 1, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is a disaccharide.

3. A composition in accordance with claim 1, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is lactose.

4. A composition for forming a glycosylated acceptor comprising an admixture of an activated glycoside derivative, a glycosyl transferase, a nucleotide phoshate sugar-dependent enzyme, an acceptor substrate and a member selected from the group consisting of a nucleotide phosphate and a nucleotide phosphate analog.

5. A composition in accordance with claim 4, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is a disaccharide.

6. A composition in accordance with claim 4, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is lactose.

7. A process for making a glycosylated acceptor, said process comprising:

admixing in an aqueous medium an activated glycoside derivative, an acceptor substrate, a glycosyl transferase, and a catalytic amount of a member selected from the group consisting of a nucleotide phosphate and a nucleotide phosphate analog, to form said glycosylated acceptor.

8. A process in accordance with claim 7, wherein said aqueous medium has a pH value of about 5 to about 10 and a temperature of about 0.degree. C. to about

40.degree. C.

9. A process in accordance with claim 7, wherein said activated glycoside derivative is a glycosyl fluoride.

10. A process in accordance with claim 7, wherein said activated glycoside derivative is a glycosyl mesylate.

11. A process in accordance with claim 7, further comprising the step of recovering said glycosylated acceptor.

12. A process in accordance with claim 7, wherein said glycosyl transferase is a member selected from the group consisting of .alpha.-sialyl transferases, .alpha.-glucosyl transferases, .alpha.-galactosyl transferases, .alpha.-fucosyl transferases, .alpha.-mannosyl transferases, .alpha.-xylosyl transferases, .alpha.-N-acetyl hexosaminyl transferases, .beta.-sialyl transferases, .beta.-glucosyl transferases, .beta.-galactosyl transferases, .beta.-fucosyl transferases, .beta.-mannosyl transferases, .beta.-xylosyl transferases, and .beta.-N-acetyl hexosaminyl transferases.

13. A process in accordance with claim 7, wherein said aqueous medium is a buffered aqueous medium.

14. A process in accordance with claim 7, wherein said acceptor substrate is selected from the group consisting of an oligosaccharide, a monosaccharide, a fluorescent-labeled saccharide and a saccharide derivative.

15. A process in accordance with claim 14, wherein said saccharide derivative is an aminoglycoside antibiotic.

16. A process in accordance with claim 14, wherein said oligosaccharide is lactose.

17. A process in accordance with claim 14, wherein said fluorescent-labeled saccharide is selected from the group consisting of an FITC-lactose, FCHASE-lactose, FITC-galactose and FCHASE-galactose.

18. A process in accordance with claim 7, wherein said activated glycoside derivative is a member selected from the group consisting of .alpha.-galactosyl fluoride, .alpha.-mannosyl fluoride, .alpha.-glucosyl fluoride, .alpha.-fucosyl fluoride, .alpha.-xylosyl fluoride, .alpha.-sialyl fluoride, .alpha.-N-acetylglucosaminyl fluoride, .alpha.-N-acetylgalactosaminyl fluoride, .beta.-galactosyl fluoride, .beta.-mannosyl fluoride, .beta.-glucosyl fluoride, .beta.-fucosyl fluoride, .beta.-xylosyl fluoride, .beta.-sialyl fluoride, .beta.-N-acetylglucosaminyl fluoride and .beta.-N-acetylgalactosaminyl fluoride.

19. A process in accordance with claim 7, wherein said glycosyl transferase is a member selected from the group consisting of .alpha.-sialyl transferases, .alpha.-glucosyl transferases, .alpha.-galactosyl transferases, .alpha.-mannosyl transferases, .alpha.-fucosyl transferases, .alpha.-xylosyl transferases, .alpha.-N-acetyl hexosaminyl transferases, .beta.-sialyl transferases, .beta.-glucosyl transferases, .beta.-galactosyl transferases, and .beta.-N-acetyl hexosaminyl transferases.

20. A process in accordance with claim 7, wherein said glycosyl transferase is immobilized on a solid support.

21. A process in accordance with claim 7, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is a disaccharide.

22. A process in accordance with claim 7, wherein said glycosyl transferase is a galactosyl transferase, said activated glycoside derivative is .alpha.-D-galactosyl fluoride and said acceptor substrate is lactose.

23. A process for forming a glycosylated acceptor by making a nucleotide phosphate glycoside in situ in a steady state concentration, said process comprising:

admixing in an aqueous medium an activated glycoside derivative, a glycosyl transferase, a member selected from the group consisting of a nucleotide phosphate and a nucleotide phosphate analog, a nucleotide phosphate sugar-dependent enzyme and at least one acceptor substrate to form a nucleotide phosphate glycoside in situ which reacts in the aqueous medium with said acceptor substrate to form said glycosylated acceptor.

24. A process for forming a glycosylated acceptor by making a nucleotide phosphate glycoside in situ in accordance with claim 23, wherein said nucleotide phosphate sugar-dependent enzyme is selected from the group consisting of a glycosyl transferase different from the glycosyl transferase in claim 23, an epimerase, a dehydrogenase, a pyrophosphorylase and a nucleotide diphosphate ribosyl transferase.

25. A process for forming a glycosylated acceptor by making a nucleotide phosphate glycoside in situ in accordance with claim 23, wherein said at least one acceptor substrate is a member selected from the group consisting of nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, glucose, a glucoside, galactose, a galactoside, mannose, a mannoside, fucose, a fucoside, N-acetylneuraminic acid, an N-acetylneuraminide, xylose, a xyloside, N-acetylglucosamine, an N-acetylglucosaminide, N-acetylgalacotosamine, an N-acetylgalactosaminide, arabinose, an arabinoside, an antibiotic aglycone, a detergent aglycone, a lipid, a sapogenin, an oligosaccharide, a monosaccharide, a fluorescent-labeled saccharide and a saccharide derivative.

26. A process for forming a glycosylated acceptor by making a nucleotide phosphate glycoside in situ in accordance with claim 23, wherein said nucleotide phosphate glycoside does not inhibit said glycosyl transferase.

27. A process in accordance with claim 23, wherein said aqueous medium has a pH value of about 5 to about 10 and a temperature of about 0.degree. C. to about 40.degree. C.

28. A process in accordance with claim 23, wherein said activated glycoside derivative is a glycosyl fluoride.

29. A process in accordance with claim 23, wherein said activated glycoside derivative is a glycosyl mesylate.

30. A process in accordance with claim 23, further comprising the step of recovering said glycosylated acceptor.

31. A process in accordance with claim 23, wherein said glycosyl transferase is a member selected from the group consisting of .alpha.-sialyl transferases, .alpha.-glucosyl transferases, .alpha.-galactosyl transferases, .alpha.-fucosyl transferases, .alpha.-mannosyl transferases, .alpha.-xylosyl transferases, .alpha.-N-acetyl hexosaminyl transferases, .beta.-sialyl transferases, .beta.-glucosyl transferases, .beta.-galactosyl transferases, .beta.-fucosyl transferases, .beta.-mannosyl transferases, .beta.-xylosyl transferases, and .beta.-N-acetyl hexosaminyl transferases.

32. A process in accordance with claim 23, wherein said nucleotide phosphate sugar-dependent enzyme is a glycosyl transferase different from the glycosyl transferase in claim 23 selected from the group consisting of .alpha.-sialyl transferases, .alpha.-glucosyl transferases, .alpha.-galactosyl transferases, .alpha.-fucosyl transferases, .alpha.-mannosyl transferases, .alpha.-xylosyl transferases, .alpha.-N-acetyl hexosaminyl transferases, .beta.-sialyl transferases, .beta.-glucosyl transferases, .beta.-galactosyl transferases, .beta.-fucosyl transferases, .beta.-mannosyl transferases, .beta.-xylosyl transferases, and .beta.-N-acetyl hexosaminyl transferases.

33. A process in accordance with claim 23, wherein said aqueous medium is a buffered aqueous medium.

34. A process in accordance with claim 25, wherein said saccharide derivative is an aminoglycoside antibiotic.

35. A process in accordance with claim 25, wherein said oligosaccharide is lactose.

36. A process in accordance with claim 25, wherein said fluorescent-labeled saccharide is selected from the group consisting of an FITC-lactose, FCHASE-lactose, FITC-galactose and FCHASE-galactose.

37. A process in accordance with claim 23, wherein said activated glycoside derivative is a member selected from the group consisting of .alpha.-galactosyl fluoride, .alpha.-mannosyl fluoride, .alpha.-glucosyl fluoride, .alpha.-fucosyl fluoride, .alpha.-xylosyl fluoride, .alpha.-sialyl fluoride, .alpha.-N-acetylglucosaminyl fluoride, .alpha.-N-acetylgalactosyl fluoride, .beta.-galactosyl fluoride, .beta.-mannosyl fluoride, .beta.-glucosyl fluoride, .beta.-fucosyl fluoride, .beta.-xylosyl fluoride, .beta.-sialyl fluoride, .beta.-N-acetylglucosaminyl fluoride and .beta.-N-acetylgalactosyl fluoride.

38. A process in accordance with claim 23, wherein said glycosyl transferase is immobilized on a solid support.

39. A process in accordance with claim 23, wherein said glycosyl transferase is a galactosyl transferase and said activated glycoside derivative is .alpha.-D-galactosyl fluoride.

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L2: Entry 6 of 6 File: USPT Feb 10, 1998

US-PAT-NO: 5716812
DOCUMENT-IDENTIFIER: US 5716812 A

TITLE: Methods and compositions for synthesis of oligosaccharides, and the products formed thereby

DATE-ISSUED: February 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CA
MacKenzie; Lloyd	Vancouver			CA
Wang; Qingping	Montreal			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
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APPL-NO: 08/ 571175 [PALM]
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US-CL-ISSUED: 435/74; 435/100, 435/172.1, 435/200, 435/201, 435/209, 536/4.1
US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209, 536/4.1

FIELD-OF-SEARCH: 435/74, 435/100, 435/172.1, 435/200, 435/201, 435/209, 536/4.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4918009	April 1990	Nilsson	435/73
☐	5246840	September 1993	Nilsson	435/101
☐	5372937	December 1994	Nilsson	435/74

FOREIGN PATENT DOCUMENTS

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0226563	June 1987	EP	
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95/18232	July 1995	WO	

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Nagashima et al. (1992) Biosci. Biotech. Biochem. 56, 207-210.
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Nikolova et al., "Transglycosylation by Wild Type and Mutants of a .beta.-1,4-Glycosidase from Cellulomonas fimi (Cex) for synthesis of Oligosaccharides", Annals NY Acad. Sci. 799: 19-25 (1996).

ART-UNIT: 184

PRIMARY-EXAMINER: Wax; Robert A.

ASSISTANT-EXAMINER: Slobodyansky; Elizabeth

ABSTRACT:

Mutant glycosidase enzymes are formed in which the normal nucleophilic amino acid within the active site has been changed to a non-nucleophilic amino acid. These enzymes cannot hydrolyze disaccharide products, but can still form them. Using this enzyme, oligosaccharides are synthesized by preparing a mixture of an .alpha.-glycosyl fluoride and a glycoside acceptor molecule; enzymatically coupling the .alpha.-glycosyl fluoride to the glycoside acceptor molecule to form a glycosyl glycoside product using the mutant glycosidase enzyme; and recovering the glycosyl glycoside product. Particular enzymes include a mutant form of Agrobacterium .beta.-Glucosidase in which the normal glutamic acid residue at position 358 is replaced with an alanine residue.

17 Claims, 3 Drawing figures

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CLAIMS:

We claim:

1. A method for synthesizing an oligosaccharide comprising the steps of:

(a) combining a glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture; and

(b) enzymatically coupling the donor molecule to the acceptor molecule using a mutant form of glycosidase enzyme to form the oligosaccharide, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of the wild-type enzyme, and said mutant enzyme being mutated to replace one of said amino acids having a carboxylic acid side chain with a different amino acid of comparable or smaller size, said different amino acid having a non-carboxylic acid side chain.

2. The method of claim 1, wherein the glycosidase enzyme is a stereochemistry retaining enzyme in which one of the carboxylic acid side chains in the active site functions as an acid/base catalyst and the other carboxylic acid side chain functions as a nucleophile, and wherein the amino acid having the nucleophilic carboxylic acid side chain is replaced in the mutant enzyme.

3. The method of claim 2, wherein the enzyme is a .beta.-glycosidase.

4. The method of claim 3, wherein the glycosyl donor molecule is an .alpha.-glycosyl fluoride.

5. The method of claim 4, wherein the .alpha.-glycosyl fluoride is an .alpha.-glucosyl fluoride.

6. The method of claim 4, wherein the .alpha.-glycosyl fluoride is an .alpha.-galactosyl fluoride.

7. The method of claim 1, wherein the enzyme is a .beta.-glycosidase.

8. The method of claim 1, wherein the enzyme is a .beta.-glucosidase.

9. The method of claim 8, wherein the enzyme is Agrobacterium .beta.-glucosidase in which amino acid 358 has been changed from glutamic acid to an amino acid with a non-carboxylic acid side chain.

10. The method of claim 8, wherein the enzyme is Agrobacterium .beta.-glucosidase in which amino acid 358 has been changed from glutamic acid to alanine.

11. The method of claim 1, wherein the acceptor molecule is an aryl-glycoside.

12. The method of claim 11, wherein the acceptor molecule is a nitrophenyl-glycoside.

13. The method of claim 1, wherein the glycosidase enzyme is a stereochemistry inverting enzyme in which one of the carboxylic acid side chains in the active site functions as an acid catalyst and the other carboxylic acid side chain functions as a base catalyst, and wherein the amino acid having the carboxylic acid side chain which functions as a base catalyst is replaced in the mutant enzyme.

14. The method of claim 1, wherein the enzyme is a mutant form of human or porcine .alpha.-amylase in which amino acid 197 has been changed from aspartic acid to alanine.

15. The method of claim 1, wherein the enzyme is a mutant form of human or porcine .alpha.-amylase in which amino acid 197 has been changed from aspartic acid to an amino acid with a non-carboxylic acid side chain.

16. The method of claim 1, wherein the enzyme is a mutant form of yeast .alpha.-glucosidase in which amino acid 216 has been changed from aspartic acid to alanine.

17. The method of claim 1, wherein the enzyme is a mutant form of yeast .alpha.-glucosidase in which amino acid 216 has been changed from aspartic acid to a non-carboxylic acid amino acid.